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Expression of immunoglobulin-cytokin fusion proteins in malignant B cells –

C L A I M S

1. Vector for the expression of immunoglobulin-cytokin fusion proteins in malignant B cells, at least containing operably linked to each other
(a) a region of at least 1.5 kb which is homologous to a region of the μ intron or the κ intron;

(b) at least one DNA sequence encoding a domain of an immunoglobulin or a part thereof;

(c) a DNA sequence encoding a cytokin; and

(d) a marker gene which is selectable in eukaryotic B cells and contains a functional enhancer region.

2. Vector according to claim 1, wherein said region of at least 1.5 kb contains a functional C_μ or C_κ enhancer.

3. Vector according to claim 1, wherein said region of at least 1.5 kb contains a non-functional C_μ or C_κ enhancer.

4. Vector according to claim 1, wherein the marker gene selectable in eukaryotic B cells contains a non-functional enhancer region.

5. Vector according to claim 1, wherein the marker gene selectable in eukaryotic B cells lacks an enhancer region.

6. Vector according to claim 1, wherein the DNA sequence of (b) encodes a constant region or a part thereof.

7. Vector according to claim 1, wherein the region homologous to a region comprising the C_μ or the C_κ enhancer of the μ or the κ intron comprises at least 1.9 kb.

8. Vector according to claim 1, wherein the region homologous to a region comprising the C_μ or the C_κ enhancer of the μ or the κ intron comprises at least 2.0 kb.

9. Vector according to claim 1, said vector containing a regulatory unit which is compatible with bacteria.

10. Vector according to claim 1, wherein the immunoglobulin is a chimeric immunoglobulin.

11. Vector according to claim 1, wherein the DNA sequence of (b) encodes the domain of a human immunoglobulin chain.

12. Vector according to claim 1, wherein the DNA sequence of (b) encodes domains derived from mouse, rat, goat, horse or sheep.

13. Vector according to claim 1, wherein the DNA sequence of

(b) encodes all the C domains of a secretory antibody.

14. Vector according to claim 1, wherein the DNA sequence according to (b) encodes all the C domains of a membrane-bound antibody.

15. Vector according to claim 1, characterized in that said DNA sequence of (c) encodes interleukins, interferons, colony-stimulating factors, lymphokins or growth factors.

16. Vector according to claim 15, characterized in that said DNA sequence of (c) encodes IL-2, IL-4, IL-7, IL-12, IL-13, GM-CSF or interferon γ.

17. Vector according to claim 1, wherein the selectable marker gene is gpt, neo or a marker gene encoding hygromycin resistance.

18. Method for the expression of an immunoglobulin-cytokin fusion protein in malignant B cells in vitro including the following steps of:
(a) introduction of a vector according to claim 1 into a malignant B cell;
(b) selection and identification of cells stably expressing the fusion protein;
(c) treatment of the cells to render them replication-incompetent.

19. Method according to claim 18, wherein step (b) is ommitted.

20. Method according to claims 18 or 19, wherein step (a) is performed by means of transfection.

21. Method according to claim 20, wherein said transfection is performed by electroporation, calciumphosphate co-

precipitation, lipofection, the DEAE dextran technique or by retroviral gene transfer.

22. Method according to claim 18, wherein the selection is carried out in a medium containing mycophenolic acid, G418, or hygromycin as a selective agent.

23. Method according to claim 18, wherein following introduction of a vector according to claim 1 by homologous recombination a site-specific integration of said vector 3´ of the heavy chain V gene of the malignant B cell is performed.

24. Method according to claim 18, wherein the expression is controlled by the endogenous V_H promoter.

25. Use of a vector according to claim 1 in the expression of immunoglobulin-cytokin fusion proteins in malignant B cells.

26. Use according to claim 25, wherein the malignant B cell is a B cell leukemia cell, a B cell lymphoma cell or a plasmacytoma cell.

27. Use according to claim 25, wherein by expression of the immunoglobulin-cytokin fusion proteins the activation of T cells is achieved.

28. Use of a vector according to claim 1 in malignant B cells for the vaccination of patients having malignant B cell diseases.

29. Malignant B cell containing a vector according to claim 1 in integrated form, wherein an immunoglobulin-cytokin fusion protein is expressed by said cell.

30. Use of a malignant B cell which has been rendered replication-incompetent and contains a vector according to claim 1 in integrated form and is capable of expression of an immunoglobulin-cytokin fusion protein in the treatment of patients having malignant B cell diseases.